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Original Article

Leptin level decreases after treatment with the combination of Radiofrequency and Ultrasound cavitation in response to the reduction in adiposity

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ABSTRACT

Background: Obesity and overweight are major public health problem. Different-strategies have been developed for body contouring including Radiofrequency(RF) and Ultrasound(US). The aim of this study was to investigate changes in serum-leptin as a potential-modulator of food/energy intake, in overweight-women receiving RF/US and diet-therapy as well as the effect of therapy on modulation of lipid-profile and body-fat-mass.

Methods: Fifty overweight-females were enrolled in current randomized-clinical-trial and randomly divided into two groups. The case group received RF/US twice a week for 5 weeks with a low calorie diet whilst the control-group received just a low calorie diet. Demographic, biochemical markers as well as serum-leptin were determined.

Results: The level of leptin was reduced from 1.29 ± 0.32 ng/ml to 1.14 ± 0.34 ng/ml in case group, before and after therapy, respectively, whilst no significant differences were observed in the serum leptin levels of subjects in the control group. The combination of RF and US decreased the leptin-level. In particular, the mean reduction of abdominal-circumference and waist-circumference was significant ($P < 0.05$) after therapy. This reduction was inversely correlated with LDL levels. Weight was reduced in case and control groups and in both was significant, but no statistically significant differences were detected for weight between the groups($P < 0.93$).

Conclusion: Our findings demonstrated the reduction of the leptin after treatment with the combination of Radiofrequency/Ultrasound cavitation, which was associated with reduced body-fat-mass.

1. Introduction

Overweight and obesity are defined as abnormal or excessive fat accumulation that may impair health.; the prevalence of obesity increasing globally (1). Body mass index (BMI) is a simple index of weight-for-height that is commonly used to classify overweight and obesity in adults. BMI <18.5 is underweight, $18.5 \leq$ BMI$\leq$24.9 normal weight, $25 \leq$ BMI$\leq$29.9 overweight and $\leq$30 is obese. Obesity and overweight are often associated with an altered lipid profile, e.g., raised serum triglycerides (TG) and low high-density lipoprotein (HDL) cholesterol levels, both are important risk factor for cardiovascular-diseases. According to World Health

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Organization (WHO), 1.1 billion adults are overweight and 312 million are obese. Obesity is associated with an increased prevalence of major health problems such as cardiovascular diseases, dyslipoproteinemia, cancer and insulin resistance [1].

It has been suggested that body weight could be regulated via hormonals released from the gastrointestinal tract, pancreas, and adipose tissue. Leptin is potentially one of the key players in this process [2], and is mainly produced by adipocytes. Leptin is a key peptide for regulation of body weight (BW), and it has been shown that the total body fat is correlated with its serum concentrations [3]. A recent study illustrated that the administration of leptin could reduce food intake. They demonstrated, for the first time, that i.n. leptin reduces appetite and induces weight loss in animal with diet induced obesity [4]. Moreover the neuroendocrine effects of leptin in the control of energy expenditure and food intake has been reported through effects on the intestine, liver, kidney, skin, stomach, heart, spleen and lung. Leptin circulates in blood and binds to its receptors in hypothalamus where it subsequently causes the release of several neuropeptides, including neuropeptide Y,that has a role in regulating energy intake and expenditure. However its role in the regulation of fat and lipid production has not been explained, but appears to be depended on modulation of energy intake, total body fat and regulation of several hormones [2].

Against this background, several strategies have been developed to manage obese people, including dietary therapy, exercise, pharmacotherapy and surgery [5]. In addition, several other non-invasive body contouring devices have been used such as Suction- Massage Devices, radiofrequency, focus ultrasound cavitation, cryolipolysis and laser therapy [6]. RF and ultrasound are the most recent technologies that have been reported as useful methods for mobilizing stored fat. RF is based on an electrical current between molecules and ions, which are then transformed into heat [7]. RF lead to the transmission of electromagnetic waves on texture and is reconstruction of old collagen, increased production of new collagen by fibroblasts stimulated, improve the quality of fibrotic septa of the adipose tissue, increased blood flow to the tissues and stimulates lipolysis. Conversely, ultrasound energy mediates a negative pressure in tissue and increases the size of air bubbles within the fat cells, causing damage to the membranes of fat cells. Thus this method has been proposed to destroy fat cells and connective tissue [6].

In the current study we aimed to explore the molecular mechanisms underlying the real influence of these 2 technologies and their effects on serum leptin level. Moreover, we evaluated the level of leptin in overweight subjects before and after therapy.

2. Materials and methods

2.1. Subjects

In the current randomized clinical study fifty women were enrolled between January 2014 and June 2014 from Nutrition/ Obesity Clinic of Ghaem Hospital, Mashhad University of Medical Sciences (MUMS). Participants were randomized into two groups (25 cases and 25 controls), using a computer-generated randomization list [8]. Exclusion criteria were: pregnant women; subjects having a history of medical illnesses such as chronic liver and/or renal diseases, and alcohol consumption, diabetes, cardiovascular disease; implantation of a pacemaker device; use of non-steroidal anti-inflammatory drugs and vitamin A and etc; women with sensitivity to light, anemia; and those who were on any weight reducing diets in last 3 months. We got fasting blood sample at the fist and end of our intervention and investigated leptin and other factors. Informed consent was collected from all participants using protocols approved by the Ethics Committee of MUMS and registered in the Iranian Registry of Clinical Trials (IRCT 2014042817475N1).

2.2. Anthropometric and biochemical parameters

BMI was calculated as body weight (kg) divided by squared height in meters (m^2), Systolic/diastolic blood pressures, height and waist circumference (WC) also determined in all the subjects followed by collecting fasting blood samples before and after intervention [8].

2.3. Lipid profile, fasting glucose measurements

Total serum cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglyceride (TG), and fasting blood glucose (FBG) concentrations were measured, as described previously [9–12].

2.4. Intervention (Ultrasound/RS)

An ultrasound device was used in the case group together with RS once per week/40 min for each cycle/10 times. US was applied at a vibration frequency of 32–36 kHz to reach a more profound impact of 6–8 cm by the Megason cavitation machine (EunSung Global Co Ltd., Seoul, Korea). The Magicpot bipolar RF (EunSung Global Co Ltd., Seoul, Korea) treatment mode was utilized at 0.8 MHz, which is recommended for deep layer, 2–4 mm treatment. Any adverse effects such as erythema, pain during treatment or blistering were recorded. Both control and case groups were received a low calorie diet for 5 weeks that consisted of a 500 kcal energy deficit per day. Subjects were advised to follow the prescribed diet plan throughout the study and all the subjects received a questionnaire to complete in follow ups to validate whether they adhered to their diet. Treatment sessions were performed twice a week by the same therapists. Controls subjects also provided a record of their diet and other parameters on 3 occasions (first, third and fifth weeks).

2.5. Measurement of leptin

The concentration of leptin was determined using a leptin enzyme-linked immunosorbent assay (ELISA) kit from LDN Labor Diagnostika Nord GmbH by an ELISA reader (Perkin Elmer X5).

2.6. Statistical analysis

Data was analyzed using SPSS-20 software (SPSS Inc., IL, USA). The normality of distributions was determined using the Kolmogorov-Smirnov test. Descriptive statistics including mean ± standard deviation (SD) were used for normally distributed variables, while median ± inter quartile (IQ) range utilized for not normally distributed variables. The t-student test was used for normally distributed data, while Wilcoxon or Mann-Whitney *U* test was used for not normally distributed variables. A statistical significance was set at $P < 0.05$.

3. Results

3.1. Baseline and clinical characteristics of case/control groups

The baseline characteristics of case and control group were reported in Table 1. Of note, no statistically significant differences were detected for age, weight, height, fat mass and BMI between the groups (Table 1). Moreover, we explored the impact of medical

Table 1
Demographic characteristics of the participants.

Variables	Case group N = 25	Control group N = 25	P value
BMI(kg/m^2)	−0.97 ± 0.4	−0.77 ± 0.36	0.09
Age (year)	36.52 ± 8.56	35.32 ± 8.70	0.625
Height (cm)	159.68 ± 4.95	161.32 ± 4.63	0.32
Weight (kg)	70.48 ± 6.24	70.44 ± 4.83	0.0.93
Medical history	1 (4)	0	0.55
Diabetes	4 (16)	5 (20.8)	
Hyperlipidemia none significant	19 (76)	19 (79.2)	
Menstrual status			0.18
Regular	22 (88)	19 (79.2)	
Irregular	3 (12)	2 (8.3)	
Menopause	0	3 (12.5)	
Education	1 (4)	2 (8)	0.664
>12 Years	12 (48)	14 (56)	
12–16 years	12 (48)	9 (36)	
<16 years			

history, menstrual status, education status between case and control groups(8). This analysis showed no significant differences between the groups in univariate analysis (Table 1).

3.2. Leptin concentration reduced after therapy

In order to explore whether there was an association between the serum leptin concentration and obesity as well the influence of therapy on leptin level, we determined the level of serum leptin in all the subjects before/after therapy (Fig. 1). As shown in Table 2, the level of leptin was decreased in the case group from 1.29 ± 0.32 ng/ml to 1.14 ± 0.34 ng/ml after US and RS (Table 2). Importantly, no significant differences in leptin concentration were detected in controls. Moreover, the mean reduction in BMI and fat mass were decreased in case group, compared to the control subjects [8], however these differences were not statistically significant. In turn, we observed that the mean reduction in abdominal circumference and waist circumference parameters was significantly greater in the case group, compared to control group, after therapy [8], which was associated with reduced level of leptin (Table 2).

3.3. Association of leptin level and LDL-C

We also evaluated the levels of LDL-C, HDL, TC and TG in our subjects before and after therapy as well as their association with leptin level. Interestingly this analysis revealed a significant inversely correlation between the level of leptin and LDL ($p = 0.03$, $r = -0.48$) in the case group (data not shown). Also it has been reported that leptin is involved in regulation of body weight and the amount of body fat is correlated with its level ([3,4,14–16]).

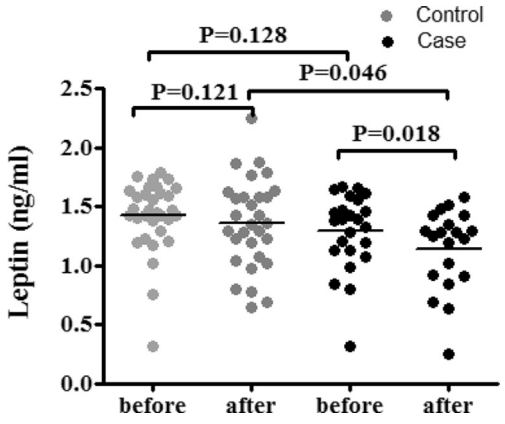

Fig. 1. Leptin level in obese subjects before/after therapy.

4. Discussion

To the best of our knowledge this is the first study evaluating the value of serum leptin as a potential modulator of food and energy intake, in overweight women receiving Radiofrequency and Ultrasound plus diet-therapiey as well as effect of these 2 methods on body-fat-mass and lipid profile. We demonstrated that the levels of leptin was high in overweight subjects and significantly reduced after treatment, which was associated with LDL level in overweight subjects.

There is growing body of evidence showing the important role of leptin in CAD and obesity. Geldszus and colleagues evaluated the serum leptin levels in 78 normal weight subjects (BMI = 16.1–27.7 kg/m2) and 149 obese women (BMI = 27.8–56.7 kg/m2). They showed that the leptin levels were increased after the end of diet therapy but remained significantly lower, compared to the control group [13]. Another recent study explored the levels of leptin in 50 overweight or obese subjects with very low energy diet. They revealed that an association between weight loss and level of leptin [2]. Rosenbaum et al. measured body composition and concentration of leptin in obese subjects. They showed that the concentration of leptin was reduced after losing of body weight [14,15]. In agreement with these observations, we observed that BMI and weight were decreased during the intervention which was associated with the reduced level of leptin in subjects that used body contouring devices. Moreover, body fat mass markedly decreased significantly followed by the reduction of leptin in case subjects after therapy, which is in line with our findings, showing a significantly relationship between the reduced level of abdomen circumference and waist circumference after therapy [8]. Also Brennan et al in 2007 demonstrated that the concentrations of leptin were correlated with the degree of body fat [16]. Additionally we observed that the level of total cholesterol, TG and LDL significantly decreased in the control group, receiving diet therapy alone, while these differences were not statistically significant in case group. Consistent with our data, several other studies indicated that this body contouring treatment does not change the level of lipid profile. Moreover, we observed that the combination of bipolar Radiofrequency energy and ultrasound cavitation technology had a significant positive effect on the reduction of abdomen and waist circumferences.

The main limitation of this study was its relatively small sample size and the absence of a group treated with RF or ultrasound alone as well as a control group who receive no intervention. Further additional analysis in a larger multi center setting is warranted to explore the value of leptin level as a sensitive marker.

5. Conclusion

In conclusion our study revealed that the level of leptin reduced after therapy, which was also associated with decreasing LDL concentration. Further studies in a larger population are needed to explore these findings.

Ethics approval and consent to participate

Informed consent was collected from all participants using protocols approved by the Ethics Committee of MUMS and registered in the Iranian Registry of Clinical Trials (IRCT 2014042817475N1).

Table 2
Serum leptin and fasted lipid profile before and after therapy.

P-value	Control		P-value	Case		Variable
	After	Before		After	Before	
0.12	1.34 ± 0.46	1.45 ± 0.25	0.01	1.14 ± 0.34	1.29 ± 0.32	Leptin(ng/ml)
0.001	181.36 ± 36.17	212.28 ± 37.35	0.15	178 ± 28.31	189.24 ± 32.61	Cholesterol(mg/dl)
0.044	93.28 ± 40.78	109.48 ± 60.10	0.35	97.04 ± 49.95	84.72 ± 47.67	TG(mg/dl)
0.001	120.76 ± 27.31	139.36 ± 30.99	0.69	112.36 ± 22.32	114.68 ± 25.88	LDL(mg/dl)
0.001	43.92 ± 12.47	50.96 ± 11.54	0.001	46.12 ± 6.11	53.60 ± 7.42	HDL(mg/dl)
0.134	79.64 ± 6.39	82.84 ± 9.45	0.984	81.80 ± 7.55	81.76 ± 7.72	FBG (mg/dl)

TC: total cholesterol; TG: triglycerides; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; FBG: fasting blood glucose.

Consent to publish

Not applicable

Availability of data and materials

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

Competing interests

The authors declare no conflict of interests.

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Authors' contributions

We declare that We contributed significantly towards the research study i.e., (a) conception, design and/or analysis and interpretation of data and to (b) drafting the article or revising it critically for important intellectual content and on (c) final approval of the version to be published.

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Abbreviations

RF	Radiofrequency
US	Ultrasound
Hs-CRP	High sensitivity C reactive protein
HDL	High density lipoprotein
LDL	Low density lipoprotein
TG	Triglycerides
WHO	World Health Organization
BW	Body weight
WC	Waist circumference
ELISA	Enzyme linked immunosorbent assay
FBG	Fasting blood glucose
MUMS	Mashhad University of Medical Science
BMI	Body mass index
SD	Standard deviation
IQR	Inter quartile range
CAD	Coronary artery disease

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